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HIV-1 Dynamics After Transient Antiretroviral Therapy: Implications for Pathogenesis and Clinical Management

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Simple models of CD4 lymphocyte interactions with human immunodeficiency virus (HIV) lead to the hypothesis that progression of HIV infection involves an increase in viral replicative capacity, due either to changes in the virus or in the host environment, or both. In order to consider how changes in plasma virus load after transient, potent antiretroviral therapy can be used to test the above hypothesis—a simple mathematical model that encompasses the processes of (1) arrival of new CD4 lymphocytes, (2) death/removal of these cells by HIV-independent mechanisms, (3) infection of susceptible CD4 lymphocytes by HIV, and (4) death/removal of infected cells was investigated. This showed that the in vivo rate of increase in plasma virus load immediately after transient therapy provides a measure of the viral replicative capacity. Thus, the hypothesis that progression of HIV infection involves an increase in viral replicative capacity can be tested by measuring this viral growth rate in patients with high CD4 counts and in patients with low CD4 counts. Studies should thus investigate dynamics of changes in virus levels after stopping antiretroviral therapy and, in particular, measure rates of increase in virus in patients at high and low CD4 counts. In practice, such data may assist in therapeutic management of patients with HIV infection. ***J. Med. Virol. 53:261–265, 1997.***

KEY WORDS: HIV infection; CD4 counts; antiretroviral therapy

INTRODUCTION

The mechanism by which infection with human immunodeficiency virus (HIV) induces gradual decline in CD4 lymphocyte numbers leading to progressive immunodeficiency, and ultimately to the development of acquired immunodeficiency syndrome (AIDS) [Lane et al., 1985; Phillips et al., 1991], is still unclear [Weiss, 1993]. Recently, others have described the rapid clearance of plasma virus, using a drug intervention approach, [Loveday, 1995; Wei et al., 1995; Ho et al., 1995] and attempted to relate this to mononuclear cell populations [Ho et al., 1995; Perelson, 1997]. In this paper, again using a therapeutic intervention approach, we develop a model for studying the other fundamental element of HIV dynamics, that of the viral replicative capacity.

We begin by considering a simple model of what is known about interactions between HIV and CD4 lymphocytes. This leads to the hypothesis that progression of HIV infection (i.e., decline in CD4 lymphocyte numbers) is caused by an increase in viral replicative capacity in the infected individual. This could be due solely to qualitative changes in the virus present or changes in the host environment (e.g., greater immune activation), or both. Having generated this hypothesis, we consider how it can be tested, by studying changes in plasma virus levels after transient, potent antiretroviral therapy.

METHODS

The model we developed is a simple version of models examined previously [e.g., McLean et al., 1991; Nowak et al., 1991; Shenzle, 1994; Essunger and Perelson,

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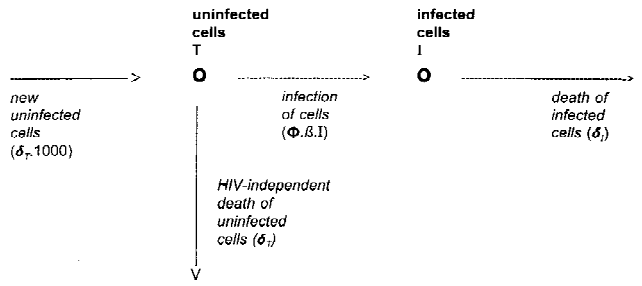

Fig. 1. Diagrammatic illustration of the mathematical model of interactions between HIV and CD4 lymphocytes. In a volume of blood or of tissue that would normally hold 1,000 CD4+ cells, T, CD4+ cells arise at rate 1,000. δ_τ, die at rate δ_τ or become infected at a rate dependent on the number of infected cells I. A fraction ϕ of CD4+ cells are activated and are therefore susceptible to productive infection, which takes place at rate β per susceptible cell per infected cell.

1994; Phillips, 1996]. We consider a quantity of virus identifiable in a CD4 lymphocyte population in either the blood or the tissue within a volume that would normally contain about 1,000 CD4 lymphocytes. This is approximately $1/(2.5 \times 10^8)$ of the entire body, in terms of lymphocytes [Phillips, 1996]. The model incorporated the following elements: First, new uninfected CD4 cells (T) arise, whether from the thymus or from division of existing cells outside the thymus, at rate $1{,}000 \cdot \delta_\tau$. Second, these cells die or are removed by HIV-independent mechanisms at rate δ_τ. A proportion of the CD4 cells (ϕ) are in the most susceptible state for productive infection (i.e., cells that are out of G_0 [Stevenson et al., 1995]; these are infected at rate $\beta \cdot I$, where I is the existing number of infected cells, and β is a more complex combination of three factors that combine to give the efficiency with which one infected cell could infect one susceptible cell. Those factors are burst size, efficiency with which free virus and infected cells infect susceptible cells, and half-life of cell-free virus. Third, infected cells are removed or die at rate δ_I.

The model is given by the following set of differential equations and is illustrated in Figure 1.

$$\frac{dT}{dt} = 1{,}000 \cdot \delta_\tau - \delta_\tau.T - \phi.\beta.I.T \qquad (1)$$

Change in number of uninfected cells	Number of new cells	Number of cells dying for HIV-unrelated reasons	Number of cells becoming infected

$$\frac{dI}{dt} = \phi.\beta.I.T - \delta_I.I \qquad (2)$$

Change in number of infected cells	Number of cells newly infected	Number of infected cells dying/removed

TABLE I. Parameter Values Used in Simulations in Figures 2–4

Parameter	Value (per day)
δ_τ	0.003
δ_I	0.5
ϕ	0.02
β	0.03

We intentionally used a simple approach in the development of this model. Interactions between HIV and its host are clearly biologically complex and multidimensional and difficult to capture in detail in any mathematical model, let alone one consisting of only two variables and two equations. The point of defining and analysing models is not, however, to try to mimic every aspect of the process under scrutiny, but to identify elements of key practical importance.

Here we investigate the above model to examine whether there are plausible circumstances under which the typical gradual CD4 lymphocyte decline seen in HIV infection would be predicted. This leads to a hypothesis that the parameter combination $\phi \cdot \beta$ gradually increases during HIV infection. This can be tested by monitoring plasma HIV-1 viral load in patients stopping potent anti-retroviral therapy.

Predicted outcomes of the model were obtained using a simulation approach known as the Euler approximation. This involves continued updating of values of T and I in steps of 0.1 days using equations (1) and (2). Parameter values used in the simulations are shown in table 1. The value for δ_τ is obtained from work on lymphocyte lifespans in uninfected individuals [McLean et al., 1995; Weng et al., 1995]. The value for δ_I has been estimated previously from observations of virus clearance rates after starting potent antiretroviral therapy [Loveday, 1995; Wei et al., 1995; Ho et al., 1995; Stellbrink, 1996]. Estimates for β are not available currently. Indeed, we are suggesting one way of generating such estimates. The value of ϕ, the proportion of CD4 cells susceptible to infection, depends on the definition of a susceptible cell. We have taken a value of 0.02 on the basis that only cells dividing or preparing to divide are susceptible to HIV infection [Stevenson et al., 1995] and that something in the region of 2% of cells are dividing at any one point in time. Variables T and I take initial values of 1,000 and 0.0000001, respectively. The latter value was chosen as it represents a whole-body inoculum of 25 cells (i.e., since we are considering $1/2.5 \times 10^8$ of the whole body). The effect of therapy was modeled by reducing the value of β to 0 for 10 days.

RESULTS

Investigation of the Model

As shown in Figure 2, numerical simulation of equations (1) and (2) predicts constant long-term levels of T and I after some initial damped oscillation. The new level of T, somewhat below the 1,000 before infection,

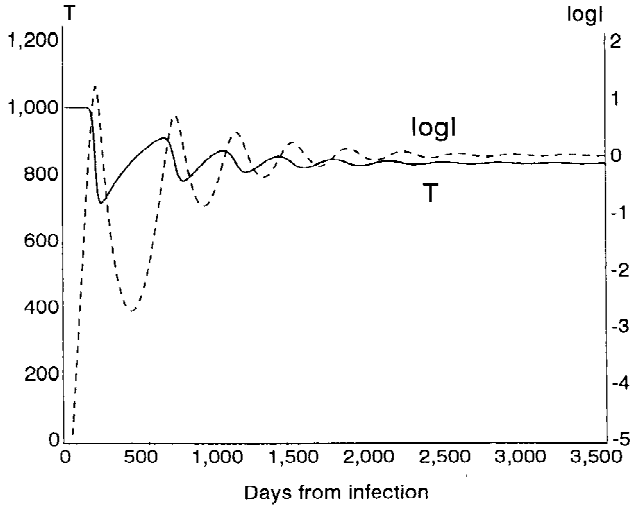

Fig. 2. If all parameter values are fixed, the model predicts constant, slightly depressed CD4+ cell numbers. Simulation of model given by equations (1) and (2) using Euler, approximation with step length 0.1 days. Parameter values are given in Table I—, T; -----, logI.

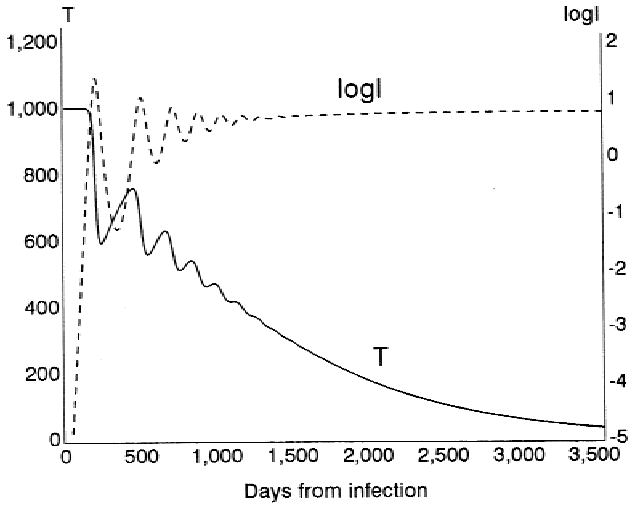

Fig. 3. If viral replicative ability slowly increases model predictions more closely approximate observed patterns. Simulation of model given by equations (1) and (2) with the added component that ϕ. β increases by a small factor with each replicative cycle. Simulation using Euler approximation with step length 0.1 days. β increases by a factor of 1.0002 per infected cell*day. β started at a value 0.03 and was 0.83 after 3,650 days (10 years). —, T; -----, logI.

reflects the fact that cells have a reduced lifespan, due to addition of an extra source of cell removal. Clearly, these are not the patterns seen in HIV infection. Rather, levels of CD4 lymphocytes, T, tend to gradually decline [Phillips et al., 1991].

The equilibrium level for T, T_{eq}, is given by setting equation (2) to zero to give

$$T_{eq} = \frac{\delta_I}{\phi \cdot \beta} \tag{3}$$

So why does T_{eq} actually appear to decline slowly during HIV infection, rather than remain constant? A decline in δ_I (i.e., a lengthening of the life span of actively infected cells and/or cell-free virus) would result in such a decline in T_{eq}, but there is evidence that such values are in fact similar in patients with low CD4 counts to those in patients with higher CD4 counts [Wei et al., 1995; Ho et al., 1995; Stellbrink et al., 1996]. This leads to the alternative that $\phi \cdot \beta$ actually increases gradually during HIV infection. This could be due to an increase in ϕ or in β, or in both.

Figure 3 shows a simulation of the model with the additional component that $\phi \cdot \beta$ increases by a very small increment for every new replicative cycle. Here, we model the increase in $\phi \cdot \beta$ as a continuous process, but it need not be the case that the increase would be continuous. For example, if an increase in β due to host-specific viral evolution is responsible for the increase in $\phi \cdot \beta$, β could increase greatly in one replicative cycle (if a useful mutation occurs), and not at all in many subsequent cycles. Figure 3 shows the result of the new simulation, which reveals a pattern of changes in CD4 lymphocyte numbers that is closer to that actually found in HIV infection.

Inferences from Transient Antiretroviral Therapy

We now consider how to test the hypothesis that $\phi \cdot \beta$ tends to be higher in patients with advanced HIV infection (i.e., low CD4 counts) than in those with earlier infection (i.e., high CD4 counts). Treatment with potent antiretroviral therapy results in an approximately 50- to 100-fold average decline in plasma HIV-1 RNA load within around 2 weeks of starting therapy [Eron et al., 1995; Markowitz et al., 1995]. If treatment is temporarily stopped at this time, when resistance is extremely unlikely to have arisen in drug-naive individuals receiving multidrug therapy, the viral changes over the next few days can be predicted from factoring out the I from equation (2). This shows that I will initially grow at a rate given by

$$\text{slope of rise in I} = \phi \cdot \beta \cdot T_0 - \delta_I \tag{4}$$

where T_0 is the CD4 count at the time of stopping therapy.

The level of cell-free virus, which is proportional to the level of cell-associated virus, will also therefore grow at this rate. On the assumption that HIV RNA load changes in plasma are representative of those throughout the body, this is the predicted rate of rise in plasma HIV RNA load in patients stopping therapy. Thus, the rate of increase in plasma HIV RNA depends on β, ϕ, the CD4 count at the time of stopping therapy and δ_I. Since all these quantities except β can be measured directly, equation (4) can be solved for β.

This suggests that an approach to testing whether β and/or ϕ increase during HIV infection would be to compare the rate of increase in virus after stopping

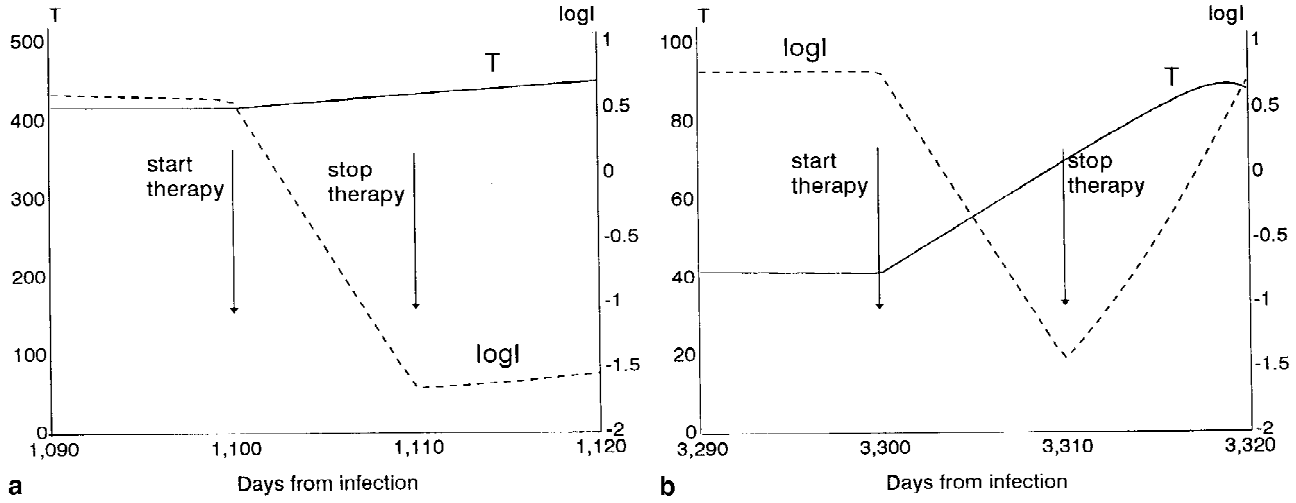

Fig. 4. Simulation using the same parameter values as that in Figure 3 showing the effect of transient (10 days) antiretroviral therapy given when the value of T is **(a)** relatively high (410), and **(b)** when the value of T is low (40). Simulation using Euler approximation with step length 0.1 days. Therapy is modeled by reducing β to zero. —, T; -----, logI.

therapy in patients with high CD4 counts and patients with low CD4 counts. If ϕ is also measured (by studying the proportion of CD4 cells that are activated/dividing), as is δ_I (by studying the rate of decline in virus when starting therapy), it is possible to estimate β and compare it between those with high and low CD4 counts.

Figure 4a,b shows simulations of the effect of starting and stopping therapy (modeled by reducing β to 0 for 10 days) on levels of T and I, if this is done when the CD4 count is relatively (a) high ($T = 410$; $\beta = 0.06$) and (b) low ($T = 40$; $\beta = 0.63$), with other parameters as they were in the model presented in Figure 3. T rises to 430 and 70 at the time therapy is stopped, respectively. The slopes of rise in virus on the natural logarithmic scale within the first few days after stopping therapy (some curvature in the line of logI can be seen after a few days of stopping therapy) are as predicted from equation (4); i.e., for (a) $\phi \cdot \beta \cdot T_0 - \delta_I = (0.02 \times 0.06 \times 430 - 0.5) = 0.016$ per day (i.e., 0.007 per day on log10 scale) and for (b) $\phi \cdot \beta \cdot T_0 - \delta_I = (0.02 \times 0.63 \times 70 - 0.5) = 0.38$ per day (i.e., 0.17 per day on log10 scale).

DISCUSSION

The rationale was outlined for undertaking a study in which patients are treated with powerful antiretroviral therapy for about 10 days before stopping therapy. A week off therapy before restarting should be enough to provide a sufficiently precise estimate of the rate of increase in virus level and by implication viral replicative capacity, as long as plasma virus concentrations are measured frequently during this period. The hypothesis to be tested is that $\phi \cdot \beta$, which is in essence a measure of the in vivo replicative capacity of virus, increases during HIV infection. If this is the case, it may well reflect the key process driving progression of HIV infection. β could increase because (1) at initial infection individuals typically receive a small inoculum, and therefore a fairly homogeneous collection of viruses; and (2) the virus enters a new host with different constraints, in terms of both target cell availability and immune surveillance, to the last host. Since there is a high level of HIV replication and enormous scope for mutations to arise [Coffin, 1995], viral evolution, reflected in an increase in β, may be expected to occur. This suggestion of an increasing β is not completely novel. Others have put forward similar ideas [Weiss, 1993; Shenzle, 1994; Essunger and Perelson, 1994; Fenyo et al., 1989; Koot et al., 1996; Zhang et al., 1993], including Shenzle [1994] and Essunger and Perelson [1994], who have presented mathematical models incorporating the concept. Nowak's model also incorporated related ideas [Nowak et al., 1990]. The idea seems plausible, although it could be considered surprising that such evolution should take as long as 10 years, unless some advantageous changes to the virus require several stepwise or simultaneous mutations. β could also increase without changes in the virus population, for example, if certain host suppressor factors preventing infection of cells were to steadily wane in effect over the course of infection. Alternatively, ϕ could increase, with a higher proportion of CD4 lymphocytes that are susceptible to infection [McLean and Nowak, 1992]. A study such as that proposed would be able to distinguish between these possibilities by measuring levels of activated cells and hence estimating β and ϕ in patients with high and low CD4 counts. Measurement of syncytium-inducing capacity would also be useful.

Our approach may be criticised for failing to explicitly model cell-free virus. We recognise that a large proportion of newly infected cells are infected by cell-free virus, rather than directly from infected cells. For simplicity, the level of cell-free virus was not modeled explicitly here, as it is assumed to be proportional to the number of infected cells (I). We have investigated a model that explicitly includes cell-free virus and the

inferences are essentially the same as those derived in this paper.

In the simulations shown, there was a great deal of initial oscillation in T and I. This has recently been observed in SIV infection in macaques undergoing daily sampling following experimental infection [Grant et al., 1997]. However, this has not yet been confirmed in clinical HIV infection, possibly due to the infrequency of sampling. We have also examined models in which a small fraction of cells initially become latently infected before subsequently becoming activated and productively infected. Such models produce much less oscillation in T and I. We elected not to include this extra complication in the model in this paper because it does not affect the main conclusions.

The study we suggest has immediate implications for patient management in addition to those for understanding pathogenesis that we have already outlined: the slope of rise in plasma HIV RNA load after stopping therapy provides an in vivo measure of the replicative capacity of viruses within that host, at that point in time, and may serve as a guide to the relative extent of therapy in any specific patient. We are in the process of testing this model by the application of different multidrug combinations.

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REFERENCES

Coffin JM (1995): HIV population dynamics in vivo: Implications for genetic variation, pathogenesis, and therapy. Science 267:483–489.

Eron JJ, Benoit SL, Jemsek J, MacArthur RD, Santana J, Quinn JB, Kuritzkes DR, Fallon MA, Rubin M (1995): Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. New England Journal of Medicine 333:1662–1669.

Essunger P, Perelson AS (1994): Modeling HIV infection of CD4+ T-cell subpopulations. Journal of Theoretical Biology 170:367–391.

Fenyo EM, Albert J, Asjo B (1989): Replicative capacity, cytopathic effect and cell tropism of HIV. AIDS 3(suppl 1):S5–12.

Grant RM, Horton CS, Rosenthal A, Dailey P, Feinberg MB, Staprans SI (1997): Plasma SIV dynamics during primary viremia. In Fourth Conference on Retroviruses and Opportunistic Infections, Washington, DC.

Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M (1995): Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection Nature 373:123–126.

Koot M, van 't Wout AB, Kootstra NA, de Goede RE, Tersmette M, Schuitemaker H (1996): Relation between changes in cellular load, evolution of viral phenotype, and the clonal composition of virus populations in the course of human immunodeficiency virus type 1 infection. Journal of Infectious Diseases 173:349–354.

Lane HC, Masur H, Gelmann EP, Longo DL, Steis RG, Chused T, Whalen G, Edgar LC, Fauci AS (1985): Correlation between immunologic function and clinical subpopulations of patients with the acquired immune deficiency syndrome. American Journal of Medicine 78:417–422.

Loveday C (1994): Quantification of serum HIV-1 RNA load by PCR. In Haeney MR (Ed): "Annual Scientific Meeting of the Association of Clinical Pathologists," October 20–21, 1994. Association of Clinical Pathologists–Yearbook 1995. London: Association of Clinical Pathologists.

Markowitz M, Saag M, Powderly WG, Hurley AM, Hsu A, Valdes JM, Henry D, Sattler F, La Marca A, Leonard JM, et al. (1995) A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. New England Journal of Medicine 333: 1534–1539.

McLean AR, Michie CA (1995): In vivo estimates of division and death rates of human T lymphocytes. Proceedings of the National Academy of Sciences USA 92:3707–3711.

McLean AR, Nowak MA (1992): Models of interactions between HIV and other pathogens. Journal of Theoretical Biology 155:69–86.

McLean AR, Emery VC, Webster A, Griffiths PD (1991): Population dynamics of HIV within an individual after treatment with zidovudine. AIDS 5:485–489.

Nowak MA, May RM, Anderson RM (1990): The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease. AIDS 4:1095–1103.

Nowak MA, Anderson RM, McLean AR, Wolfs TF, Goudsmit J, May RM (1991): Antigenic diversity thresholds and the development of AIDS. Science 254:963–969.

Perelson AS (1997): Viral and cellular dynamics: Implications for antiretroviral therapy. In Fourth Conference on Retroviruses and Opportunistic Infections, Washington, DC.

Phillips AN, Lee CA, Elford J, Janossy G, Timms A, Bofill M, Kernoff PB (1991): Serial CD4 lymphocyte counts and development of AIDS. Lancet 337:389–392.

Phillips AN (1996): Reduction of HIV concentration during acute infection: independence from a specific immune response. Science 271:497–499.

Shenzle D (1994): A model for AIDS pathogenesis. Statistics in Medicine 13:2067–2079.

Stellbrink HJ, Zoller B, Fenner T, Lichtenberg G, Van Lunzen J, Albrecht, Thiele H, Greten H (1996): Rapid plasma virus and CD4+ T-cell turnover in HIV-1 infection: evidence for an only transient interruption by treatment. AIDS 10:849–857.

Stevenson M, Brichacek B, Heinzinger N, Swindells S, Pirruccello S, Janoff E, Emerman M, (1995): Molecular basis of cell cycle dependent HIV-1 replication. Implications for control of virus burden. Advances in Experimental Medicine and Biology 374:33–45.

Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, Lifson JD, Bonhoeffer S, Nowak MA, Hahn BH, et al. (1995): Viral dynamics in human immunodeficiency virus type 1 infection. Nature. 373:117–122.

Weiss RA (1993): How does HIV cause AIDS? Science 260:1273–1279.

Weng N, Levine BL, June CH, Hodes RJ (1995): Human naive and memory T lymphocytes differ in telomeric length and replicative potential. Proceedings of the National Academy of Sciences, USA 92:11091–11094.

Zhang LQ, MacKenzie P, Cleland A, Holmes EC, Leigh Brown AJ, Simmonds P (1993): Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. Journal of Virology 67:3345–3356.